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Letter to the Editor

Thermal Enhancement of Bleomycin-induced Growth Delay in a Squamous Carcinoma of CBA/Ht Mouse*

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A CONSIDERABLE number of reports have appeared in recent years (e.g. [1–4]) describing enhanced cytotoxicity of chemotherapeutic agents by hyperthermia, principally in mammalian cells in culture. Suggested explanations of the enhancement include increased rates of drug metabolism to form an active intermediate [1], changes in plasma membrane permeability [4] and depression of repair of potential lethal damage [2]. Relatively few reports involving *in vivo* studies have appeared. Hahn [3] indicated that *in vivo* studies involving animal tumour systems in his lab have shown that those agents which show synergism *in vitro* also do so *in vivo*.

The experiments described in this paper demonstrate that heat-induced potentiation of drug effects can be achieved in solid murine tumours under certain conditions.

Experiments were performed using inbred CBA/Ht mice, 4 weeks old, with an isogeneic squamous cell carcinoma, CBA Sq Ca 1, whose growth characteristics in these inbred mice have been listed by Hewitt *et al.* [5]. The tumour was implanted intradermally or subcutaneously midway between and in line with front and rear limbs, to allow hyperthermia treatment without direct heat of the peritoneal cavity. Tumour diameter was measured regularly using graduated calipers, once the tumour became palpable at approximately 7 days.

Growth curves for each individual were constructed and the time taken to double tumour diameter, the slope of the linear portion of growth curve and the time taken to reach 15 mm were calculated after normalising initial tumour diameter to control values. The median values for these parameters were calculated from individual figures and for two of the three parameters above the standard deviation from the median value calculated. Individual values in each group were compared to the groups of values for the controls using Student's '*t*' test to determine the significance of the difference in median value between the test and control groups.

Growth delay is defined as the increased time taken to reach 15 mm in diameter from a normalised initial diameter and is also incorporated in the concept of tumour diameter doubling time. Growth rate is limited to the description of the magnitude of increase in diameter in the linear portion of growth after the period of initial suppression of growth by the treatment has passed.

Hyperthermic conditions were achieved by localised exposure to 0.75 MHz ultrasound for 45 sec at 1.5 W/cm^2 followed by continuous exposure at 0.5 W/cm^2. Temperature profiles were obtained by insertion of the tip (0.8 mm) of a hypodermic thermocouple attached to a Digitron 175-K digital thermometer at different depths and positions in the tumour. At least six readings per tumour were taken. The initial temperature in the tumour of the anaethetised animal of approximately 34°C rose to 43°C during the first 45 sec at 1.5 W/cm^2 and remained at this value ±0.2°C during the period of subsequent exposure at 0.5 W/cm^2. The variation reflects the difference in temperature dependent on the depth at which the reading was taken.

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The results in Table 1 indicate that radiolabelled bleomycin was approaching maximal tumour to blood values after ten minutes, whilst in the anaethetised animal this condition did not occur until at least twenty minutes after i.p. injection. The same total activity of radiolabelled bleomycin was injected into individuals in both groups, so the results reflect the longer time taken to reach maximal tumour to blood values in the anaethetised animal. Hence simultaneous treatment of tumours with bleomycin and hyperthermia in anaethetised animals required the drug to be injected i.p. more than twenty minutes before exposure to heating conditions.

Bleomycin (50 mg/kg) produced a significant delay in the tumour doubling time resulting from an enhanced delay before growth resumed. There was no significant difference in growth rate from the controls once growth had recommenced, as assessed over the linear portion of the curve. Hyperthermia (43°C/15 min) significantly influenced both parameters (Table 2).

The influence of combined drug and heat treatment was considered in relation to the sum of the individual effects (Table 2). The results suggest that potentiation of drug effects are obtained only when both treatments are applied simultaneously.

Median values for the time taken to reach 15 mm are reported in Table 3. The Dose Modifying Factor (D.M.F.) is defined and again reflects the fact that significant potentiation

Table 1. Uptake of [^{57}Co]-bleomycin into blood and tumour of mouse as a function of time

Time after [^{57}Co]-BLM injection (min)	Normal conditions		Under anaesthetic	
	Blood (μg/ml)	Tumour (μg/100 mg)	Blood (μg/ml)	Tumour (μg/100 mg)
2	2.4	0.09	0.09	0.003
10	20.7	0.97	0.15	0.007
20	21.5	1.04	8.1	0.56
30			16.3	1.09
40			21.6	1.16

[^{57}Co]-BLM, 10 μCi/μM. Bleomycin injection i.p., 50 mg/kg.

Table 2. Median doubling times and growth rates of tumour under a variety of conditions

Group	No. of animals per group	Median time (days) to double tumour diameter ±S.D.	Significance (Student's '*t*') compared to control	Mean slope (mm/day) linear portion of curve ±S.D.	Significance (Student's '*t*') compared to control
Control	4	8.6 ± 0.8	—	0.78 ± 0.03	—
Bleomycin (25 mg/kg)	4	9.9 ± 0.5	N.S.	0.83 ± 0.03	N.S.
Bleomycin (50 mg/kg)	4	13.2 ± 0.9	Sig.	0.80 ± 0.02	N.S.
Hyperthermia (43°C/7 min)	4	9.0 ± 0.3	N.S.	0.80 ± 0.01	N.S.
Hyperthermia (43°C/15 min) 43°C/15 min)	4	11.5 ± 0.5	Sig.	0.70 ± 0.03	Sig.
Pre-heat (43°C/15 min + 25 mg/kg BLM)	4	11.6 ± 1.2	*	0.67 ± 0.03	*
Pre-heat (43°/15 min + 50 mg/kg BLM)	4	19.0 ± 0.2	*	0.74 ± 0.01	*
Simultaneous (25 mg/kg BLM + 43°C/15 min)	4	16.7 ± 0.5	***	0.56 ± 0.02	***
Simultaneous (50 mg/kg BLM + 43°C/15 min)	4	24.2 ± 0.6	***	0.46 ± 0.01	***

Combined treatment: *additive or less than additive; ***greater than additive.

Table 3. Dose-modifying factors based on the potentiation of drug action by hyperthermia treatment

	Time to reach 15 mm tumour diameter median value (days)	Dose-modifying factor (D.M.F)*
Control	10.7	—
Bleomycin (25 mg/kg)	12.4	—
Bleomycin (50 mg/kg)	15.9	—
Hyperthermia (43°C/15 min)	14.2	—
Hyperthermia followed by BLM (25 mg/kg)	15.1	0.90
Hyperthermia followed by BLM (50 mg/kg)	22.2	1.32
BLM (25 mg/kg) + hyperthermia simultaneous	20.8	1.94
BLM (50 mg/kg) + hyperthermia simultaneous	29.3	2.14

*D.M.F. = $\frac{\text{Observed delay}}{\text{Sum of delays of individual treatments}}$ compared to control.

may only be observed for simultaneous treatment.

Rate of uptake of ^{57}Co-bleomycin was studied in a culturable cell line (HeLa) rather than in cells of the squamous cell carcinoma. However, the results (Table 4) are of interest as they suggest that the enhanced effects of simultaneous treatment of growth delay and growth rate may not result from enhanced permeability to drug uptake. Results published by Braun and Hahn [6] for a different cell line support this conclusion.

Table 4. [^{50}Co]-BLM accumulation in HeLa cells during and post hyperthermia

Experimental conditions	BLM × 10^{-9} gm/10^6 cells ±S.D.
[^{57}Co]-Bleomycin 37°C	286 ± 12
[^{57}Co]-Bleomycin 43°C	36 ± 4
Incubation at 43°C Treated with bleomycin subsequently at 37°C	30 ± 3

Heating time 90 min; bleomycin concentration, 100 μg/ml; specific activity [^{57}Co]-BLM, 8 μCi/μM.

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